

A method for treatment of Pythiosis in human patients having the Pythiosis which comprises:

(a) providing a vaccine containing a mixture of mixed intracellular proteins and mixed extracellular proteins of *Pythium insidiosum* in a sterile aqueous solution, wherein the mixed intracellular proteins, which consist essentially of proteins removed from disrupted cells of the *Pythium insidiosum* grown in a culture medium, and the mixed extracellular proteins, which consist essentially of proteins removed from the culture medium for growing the *Pythium insidiosum*, are in water and the mixture has been dialyzed to remove low molecular weight components less than 10,000 MW; and

(b) vaccinating the patient with the vaccine.

The method of Claim 16 wherein vaccinating the patient with the vaccine is subcutaneous.

A method for the treatment of Pythiosis in a mammal having the Pythiosis which comprises:

5 (a) providing an injectable vaccine derived from growing cells of *Pythium insidiosum* in a culture medium which comprises in a sterile aqueous solution in admixture:

10 (1) mixed intracellular proteins, which consist essentially of proteins removed from disrupted cells of the *Pythium insidiosum* separated from the culture medium; and

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(2) mixed extracellular proteins, which consist essentially of proteins removed from the culture medium separated from the cells of the *Pythium insidiosum*;

15 wherein the admixture in water has been dialyzed to remove low molecular weight components less than 10,000 MW to produce the vaccine; and

(b) vaccinating the mammal with the vaccine.

The method of Claim 18 wherein the removed proteins in the admixture have been provided by growing cells of the *Pythium insidiosum* in the culture medium, then killing the cells, then separating the killed cells from the culture medium to produce a first supernatant to provide the mixed extracellular proteins of (a)(2) and then disrupting the killed cells in sterile water and removing the disrupted cells from the sterile water containing the mixed intracellular proteins to provide the mixed intracellular proteins of (a)(1) in a second supernatant, combining the first and second supernatants, precipitating the proteins, resuspending the precipitated proteins in sterile water, and dialyzing the resuspended proteins in sterile water to remove the material less than 10,000 MW.

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cont

The method of Claim 19 wherein the disrupted cells are removed from the sterile water containing the mixed intracellular proteins by centrifugation to provide the mixed intracellular proteins of (a)(1) in the second supernatant.

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-25-(Fourth amended)

The method of Claim 19 wherein the mixed intracellular and extracellular proteins from (a) (1) and (a) (2) are precipitated with acetone to produce a precipitate and resuspending the precipitate in sterile distilled water for the dialysis.

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